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TECHNICAL MANUSCRIPT 501

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Charles P. P. Reid
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MAY 1969

DEPARTMENT OF THE ARMY
Fort Detrick
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TECHNICAL MANUSCRIPT 501

ROOT PERMEABILITY AS AFFECTED BY PICLORAM AND OTHER CHEMICALS

Charles P.P. Reid

Woodland Hurtt

Plant Physiology Division
PLANT SCIENCES LABORATORIES

Project 1B562602A061 May 1969

ABSTRACT

Because increased water deficit in the plant caused by changes in root permeability can result in transpiration reduction, the effects of picloram (4-amino-3,5,6-trichloropicolinic acid) and several other chemicals on root permeability were studied. Initially, effects on cell permeability were investigated by measuring the betacyanin efflux from red beet (Beta vulgaris L.) root sections. Picloram solutions ranging from 10^{-3} M to 10^{-6} M had no significant effect on betacyanin efflux when compared with controls. Similar results were found for 10^{-4} M and 10^{-5} M 2-methoxy-3,6-dichlorobenzoic acid (dicamba), 10^{-4} M 2,4-dichlorophenoxyacetic acid (2,4-D), and 10 ppm of ethylene. Compounds that caused significant pigment leakage were 10^{-4} M and 10^{-5} M phenylmercuric acetate, 10^{-3} M and 10^{-4} M 2,4-dinitrophenol, 10^{-3} M and 10^{-4} M 2,4,5-trichlorophenoxyacetic acid, and 10^{-3} M 2,4-D.

Further investigations were conducted with picloram applications to the roots of bean plants (Phaseolus vulgaris L. var. Black Valentine) grown in nutrient solution under controlled environmental conditions. Roots treated for 3 hours with 10^{-5} M picloram showed no significant electrolyte leakage as determined by conductivity measurements of the root bathing solution for 52 hours. When bean roots were treated for 3 hours with 10^{-5} M, 10^{-6} M, and 10^{-7} M picloram and then the plants detopped, increased stem exudation was observed. Xylem exudate of the treated plants also showed increased electrolytic conductivity. The increased exudation rate accompanied by increased conductivity indicates that picloram has little effect on root cell membrane integrity, does not act as a metabolic inhibitor, and in some way stimulates salt secretion into the xylem.

I. INTRODUCTION*

Picloram (4-amino-3,5,6-trichloropicolinic acid) is a highly biologically active herbicide with apparently unique properties. It causes distortions in growing tissue and other formative effects[1] and growth promotion in stem sections, similar to that caused by 2,4-dichlorophenoxyacetic acid (2,4-D) and other auxin-like growth regulators.[2,3] Picloram has also been characterized as comparable to 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in absorption and translocation.[1] To further elucidate the modes of action of picloram, studies have been conducted on its effect on foliar transpiration. Because increased water deficit in the plant caused by changes in root permeability can result in transpiration reduction, the effects of picloram and several other chemicals on root permeability were studied.

II. MATERIALS AND METHODS

A technique of Veldstra and Booij[4] involving the measurement of betacyanin efflux from red beet root sections was used for investigating the possible effects of selected substances on cell permeability. Discs (10 mm by 3 mm) were cut from the roots of red beet (*Beta vulgaris* L.), rinsed several times in distilled water, and then retained in distilled water overnight at 8 C. After again rinsing, 10 discs were placed in Erlenmeyer flasks containing 50 ml of solution. Each of the following compounds was prepared in 0.5X Hoagland's nutrient solution and replicated three times: picloram, 10^{-3} M, 10^{-4} M, 10^{-5} M, and 10^{-6} M; 2,4-D, 10^{-3} M and 10^{-4} M; 2,4,5-T, 10^{-3} M and 10^{-4} M; 2-methoxy-3,6-dichlorobenzoic acid (dicamba), 10^{-4} M and 10^{-5} M; phenylmercuric acetate (PMA), 10^{-4} M and 10^{-5} M; 2,4-dinitrophenol (DNP), 10^{-3} M and 10^{-4} M; and ethylene, 10 ppm. All solutions were adjusted to pH 6.05±0.12.

Pigment release from the discs was determined by measuring the light absorbancy of the solution at 500 mμ with a Bausch & Lomb Spectronic 20 colorimeter and comparing the values with blanks of 0.5X Hoagland's solution. Measurements were made periodically up to 24 hours; the flasks were maintained at 25 C.

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To study the effect of picloram on exudation rate, bean plants (<u>Phaseolus vulgaris</u> L. var. Black Valentine) were grown in nutrient culture in a controlled environment growth chamber with a 16-hour photoperiod provided by 1,400 ft-c of illumination at plant-top level. Temperature and relative humidity were maintained at 25±1 C and 50±5%, respectively. When the first compound leaves were beginning to unfold, plant roots were treated for 3 hours with 300 ml of selected molarities of picloram in 0.5X Hoagland's nutrient solution. After treatment, each plant was transferred to a 250-ml Erlenmeyer flask containing aerated distilled water. Control plants were manipulated in the same manner as the treated plants except that picloram was omitted. Each series of picloram-treated plants and control plants consisted of 10 replications. To measure exudation rate, the top of each plant was removed 2 cm below the cotyledonary node immediately after the 3-hour treatment period, and a 0.2-ml graduated pipette was attached vertically to the cut stem by a short length of rubber tubing. At the start of each measurement period, the pipette was adjusted to the zero mark by injecting distilled water containing acid fuschin dye. The increase of volume in the pipette caused by xylem exudate was then recorded periodically and expressed as milliliters of exudate per hour.

Electrolyte conductivity of the root bathing solution was measured in 5-ml samples removed periodically from the flasks containing treated and control plants. Solution conductivity (μmhos at 25 C) was determined with an Industrial Instruments Inc. Conductivity Bridge Model RC16B2 using a 3-ml pipette cell with a 0.1 cell constant.

Conductivity of xylem exudate from several different experiments was also determined. Plants were treated as above but exudate was collected in 5-ml plastic containers attached to each stem. Eleven collections were made, each consisting of the pooled exudate from at least six plants. The conductivity of a particular exudate collection from plants treated with 10^{-5} M picloram was compared with the control plant exudate conductivity at the same collection period. A statistical analysis based on paired observations[5] was then performed on all collections.

III. RESULTS AND DISCUSSION

The effects of picloram and the other compounds on cell permeability are shown in Figure 1. The degree of disturbance of the semipermeability of the membranes of the beet root cells is indicated by the absorbancy value of the bathing solution, with higher values demonstrating greater leakage of betacyanin from the vacuoles. The loss of pigment was greatest with 10^{-4} M PMA followed in descending order of loss by 10^{-3} M 2,4,5-T, 10^{-3} M DNP, 10^{-5} M PMA, 10^{-3} M 2,4-D, 10^{-4} M 2,4,5-T, and 10^{-4} M DNP. No significant loss of pigment over the 24-hour period was detected for 10^{-3} M, 10^{-4} M, 10^{-5} M, and 10^{-6} M picloram; 10^{-4} M 2,4-D; 10^{-4} M and 10^{-5} M dicamba; or 10 ppm of ethylene. The slight peak observed for ethylene at 2 hours is thought to be the result of a spurious reading caused by air bubbles in the solution.

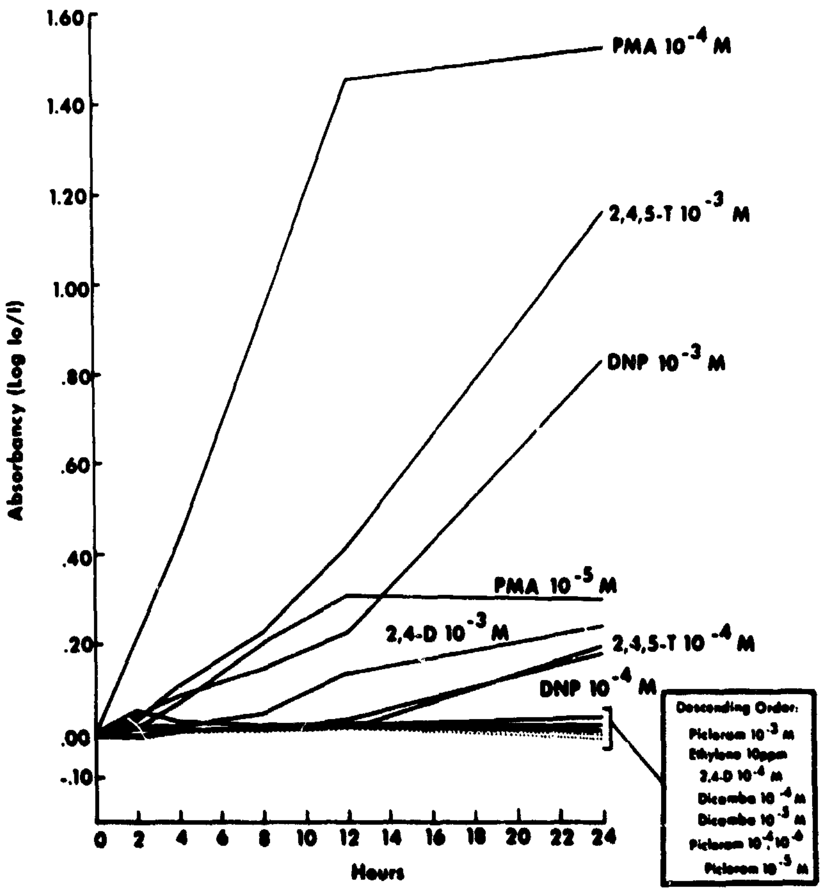

FIGURE 1. Effects of Various Compounds on Pigment Efflux from Beet Root Discs at 25 C. Degree of efflux refected as absorbancy value of solution. All values above 0.06 are significantly different from the control at 5% level.

In addition to picloram, 2,4-D, 2,4,5-T, and dicamba were tested because of their known effectiveness as herbicides. The responses observed with 2,4-D and 2,4,5-T are similar to those reported by Veldstra and Booij.[4] They found that the chlorinated compound 2,4,6-trichlorophenoxyacetic acid interacted with beet membranes more strongly than 2,4-D. This stronger response was attributed to the increase of the lipophilic component of the molecule by the introduction of the added chlorine atom.

DNP, a metabolic inhibitor known to affect membrane permeability adversely, was included as a basis for comparison. Surprisingly, PMA proved to be the most effective compound in causing pigment leakage, even at a lower concentration than DNP. PMA has been studied extensively as an antitranspirant and fungicide.[6] Siegenthaler and Packer[7] found that PMA inhibits light-induced chloroplast swelling and also inhibits nicotinamide adenine dinucleotide phosphate reduction and photophosphorylation reactions. Perhaps the effect of PMA on regulation of chloroplast volume is also related to changes in membrane permeability. Ethylene, a gaseous plant growth regulator,[8] has been considered to cause alteration to membrane permeability, although no such effect appeared under the particular experimental conditions used here.

It is particularly interesting that picloram had no appreciable effect on membrane permeability as compared with 2,4-D and 2,4,5-T, because picloram is often compared with these compounds as having similar growth regulation characteristics. Veldstra and Booij[4] noted that the activity of compounds such as indoleacetic acid, naphthaleneacetic acid, and 2,4-D, as determined by the beet disc test, reflects very well their phytotoxicity as determined from studies on parts of plants or intact plants. Obviously, this is not the case for picloram.

Exudation rates resulting from treatment with picloram further support the thesis that picloram does not affect membrane permeability adversely. Increases in exudation rate were consistently observed for plants treated with 10^{-5} M, 10^{-6} M, and 10^{-7} M picloram (Fig. 2 to 4). Increases in exudation rate were significant 7 hours after the initiation of treatment for the 10^{-5} M and 10^{-6} M picloram-treated plants and remained significant throughout the experiments. Exudation rate of the 10^{-7} M picloram-treated plants was not significantly greater during the first 7 hours but was significant after 22 hours. Because exudation rate from detopped plants is primarily affected by salt transfer into the xylem,[9-11] it appears that picloram directly or indirectly causes an increased movement of salt into the xylem. In fact, statistically higher conductivities, indicating a greater salt concentration, occurred in the collected exudate from plants treated with 10^{-5} M picloram when compared with controls (Table 1).

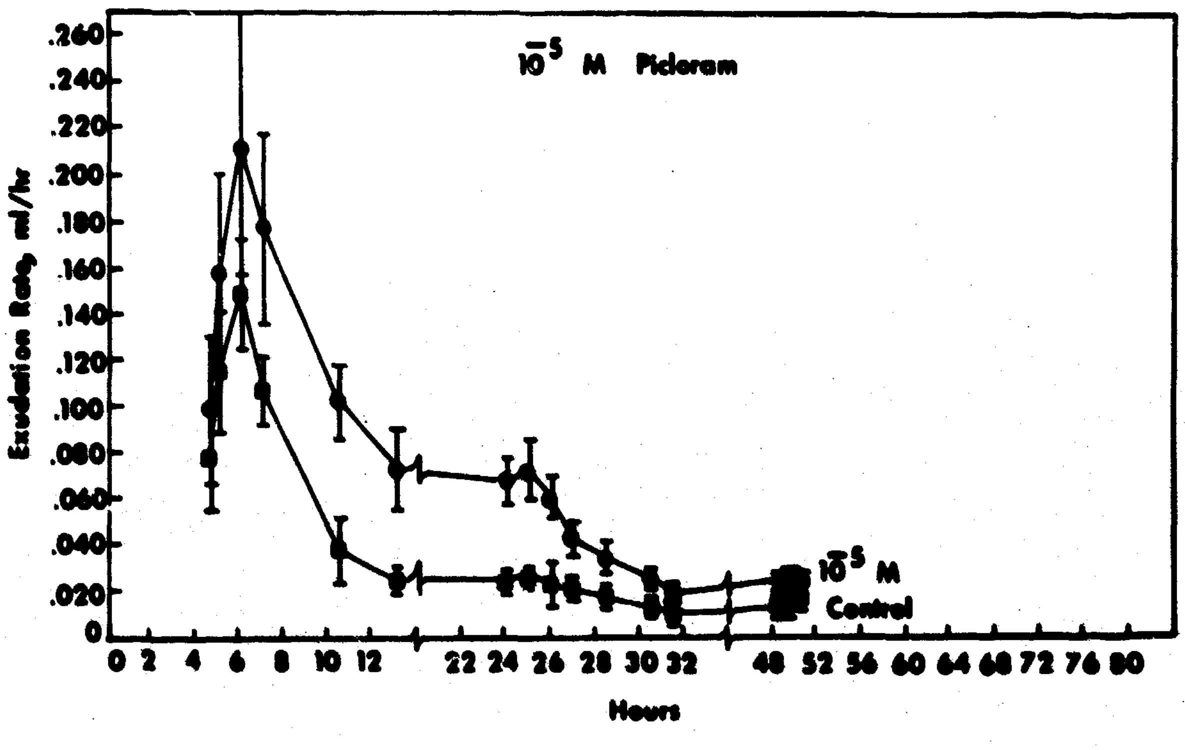

FIGURE 2. Effect of 10^{-5} M Picloram on Exudation Rate of Detopped Plants. Three-hour picloram treatment on roots initiated at zero time. Vertical bars represent 95% confidence limits on the mean.

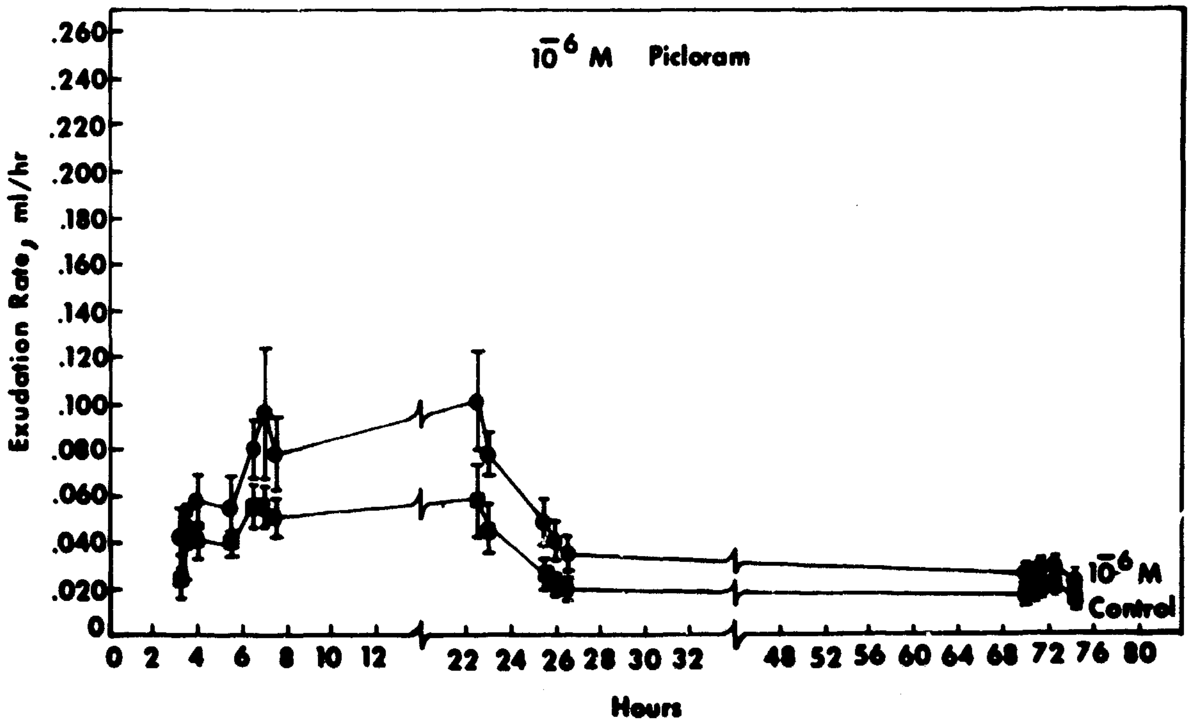

FIGURE 3. Effect of 10^{-6} M Picloram on Exudation Rate of Detopped Plants. Three-hour picloram treatment on roots initiated at zero time. Vertical bars represent 95% confidence limits on the mean.

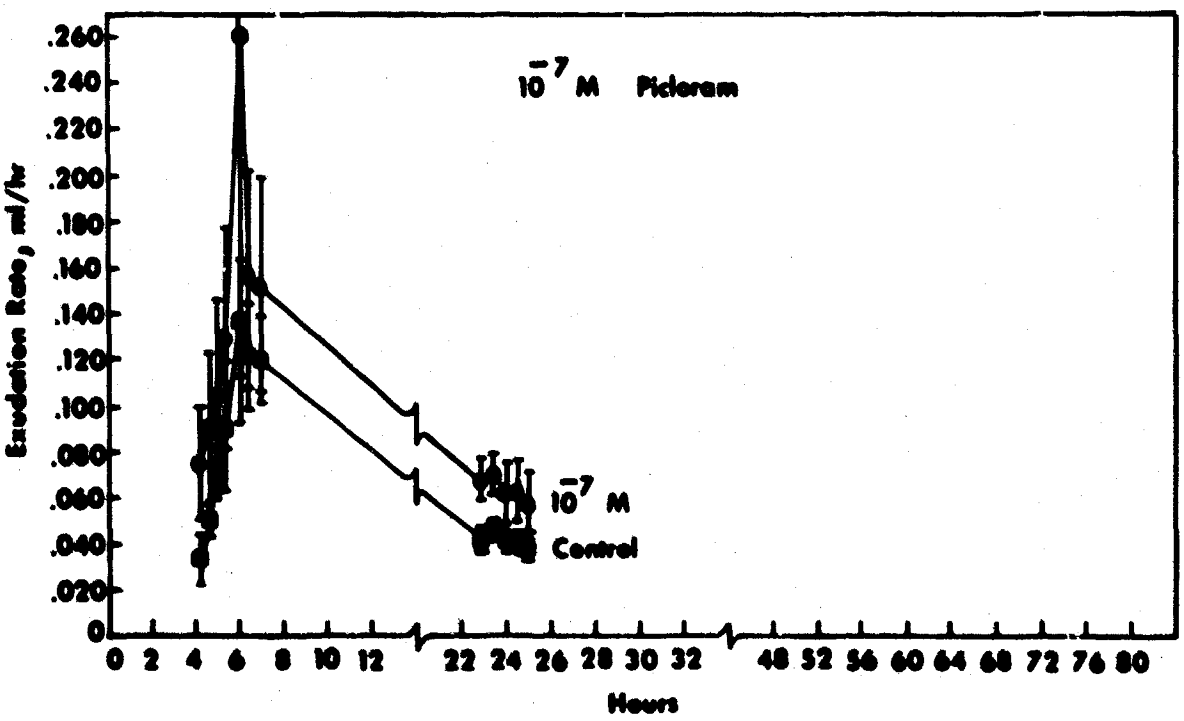

FIGURE 4. Effect of 10^{-7} M Picloram on Exudation Rate of Detopped Plants. Three-hour picloram treatment on roots initiated at zero time. Vertical bars represent 95% confidence limits on the mean.

TABLE 1. COMPARISON OF XYLEM EXUDATE CONDUCTIVITY FROM PICLORAM-TREATED PLANTS AND CONTROLS

Treatment	Conductivity, µmhos, for Indicated Exudate Collection Number[a]											
	1	2	3	4	5	6	7	8	9	10	11	Mean
10^{-5} M Picloram	1,140	2,325	1,950	2,720	980	1,160	2,530	2,260	2,180	2,260	2,200	1,973
Control	600	1,725	1,725	1,910	995	745	2,250	2,250	2,120	2,080	1,860	1,660
Difference	540	600	225	810	(15)	415	280	10	60	180	340	313[b]

a. Each figure represents the pooled exudate from at least six plants.
b. Statistically significant at 1% level using paired observations analysis.[5]

Salt moves into the xylem even though the roots are bathed in distilled water,[9,11] as was done here. However, the exudation rate steadily declines with time (Fig. 2 to 4).

Wallace et al.[11] indicate that movement of salt into the xylem appears to be under metabolic control. Considering that inhibitors such as DNP and KCN decrease exudation rate,[10] it seems very unlikely that picloram acts as a metabolic inhibitor in the root system.

The measurements of the conductivity of the root bathing solution of the plants treated with 10^{-5} M picloram indicated no statistically significant electrolyte loss as compared with controls (Fig. 5). The increase of exudation rate with no change in ion leakage from the roots seems to be in agreement with the results of Newman and Kramer.[12] In the reverse situation, they found that a decrease in root pressure exudation accompanied an increase of electrolyte leakage from roots immersed in decenylsuccinic acid, a metabolic inhibitor.

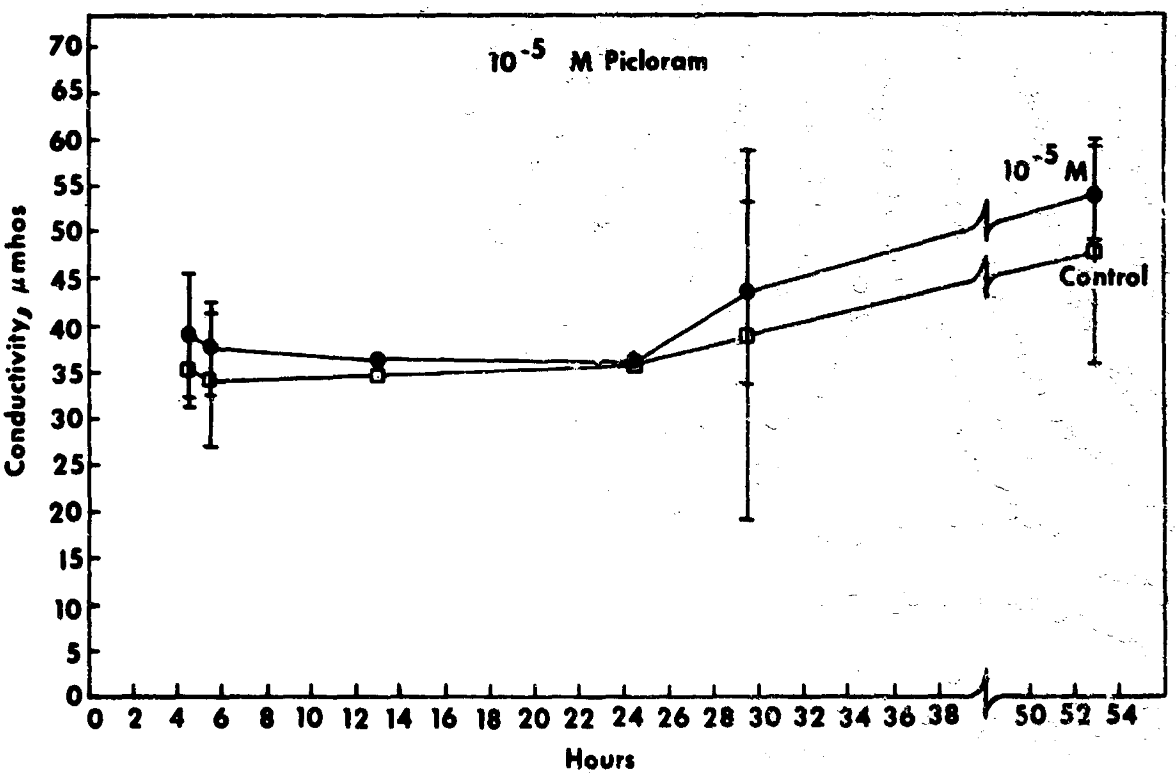

FIGURE 5. Conductivity of the Root Bathing Solution of Plants Treated for 3 Hours with 10^{-5} M Picloram. Vertical bars represent 95% confidence limits on the mean.

Although it is not known why exudation rate is increased by picloram, it might be explained as an auxin-like effect. It has been demonstrated that auxin in low concentrations causes increased exudation,[13] and that auxin causes increased water uptake by plant tissues.[14] However, it is generally agreed that auxin-induced water uptake is primarily related to effects on cell wall extensibility, which in turn permits osmotic water uptake, rather than effects on membrane permeability per se.[14]

The increased salt concentration found in the xylem exudate cannot be explained entirely on the basis of an auxin effect, and for this reason another mechanism appears to be involved other than wall extensibility, perhaps one affecting ion transport across membranes.

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13. ABSTRACT Because increased water deficit in the plant caused by changes in root permeability can result in transpiration reduction, the effects of picloram (4-amino-3, 5,6-trichloropicolinic acid) and several other chemicals on root permeability were studied. Initially, effects on cell permeability were investigated by measuring the betacyanin efflux from red beet (*Beta vulgaris* L.) root sections. Picloram solutions ~~ranging from 10^{-3} M to 10^{-6} M~~ had no significant effect on betacyanin efflux when compared with controls. Similar results were found for [redacted] 2-methoxy-3, 6-dichlorobenzoic acid (dicamba), [redacted] 2,4-dichlorophenoxyacetic acid (2,4-D) and 10 ppm of ethylene. Compounds that caused significant pigment leakage were [redacted] and [redacted] phenylmercuric acetate, [redacted] and [redacted] 2,4-dinitrophenol, [redacted] and [redacted] 2,4,5-trichlorophenoxyacetic acid, and [redacted] 2,4-D.

Further investigations were conducted with picloram applications to the roots of bean plants (*Phaseolus vulgaris* L. var. Black Valentine) grown in nutrient solution under controlled environmental conditions. Roots treated for 3 hours with [redacted] picloram showed no significant electrolyte leakage as determined by conductivity measurements of the root bathing solution for 52 hours. When bean roots were treated for 3 hours with [redacted], [redacted], and [redacted] picloram and then the plants detopped, increased stem exudation was observed. Xylem exudate of the treated plants also showed increased electrolytic conductivity. The increased exudation rate accompanied by increased conductivity indicates that picloram has little effect on root cell membrane integrity, does not act as a metabolic inhibitor, and in some way stimulates salt secretion into the xylem.

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